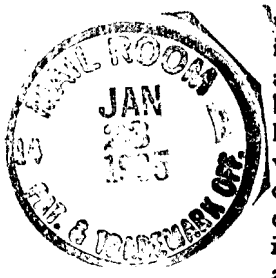
EX-1

Monoclonal antibodies: clinical and regulatory issues

meeting report



The potential for the *in vivo* clinical use of monoclonal antibodies (mAbs) is now firmly acknowledged, although successful applications may be more limited than was originally hoped. The aim of a recent meeting* was to review the current situation concerning the useful clinical applications of various types of mAbs, including consideration of the desirable (and undesirable) consequences of their clinical use and highlighting the regulatory issues that have proven either problematical or contentious.

Shock states

Following a brief introduction to the available mAb technologies and production routes [R. Thorpe, National Institute for Biological Standards and Control (NIBSC), Herts., UK], the meeting started with a session devoted to the treatment of shock states. A. Gearing (British Bio-technology, Oxford, UK) reviewed the pathology, identifying some potential targets and highlighting the most recent hypotheses concerning adhesion-molecule involvement in shock states. R. Stroube (Centocor, Malvern, PA, USA) then described the characteristics and clinical effects of HA-1A ('Centoxin'), a human IgM anti-lipid A mAb, which has been used fairly extensively for treatment of Gram-negative bacteraemia with septic shock. HA-1A binds to rough mutants of Gram-negative organisms, but less well to smooth strains, although treatment with cell-wall-active antibiotics improves reactivity with the latter. The mAb also binds to bacterial fragments as well as LPS isolated from organisms. The mechanism by which HA-1A functions clinically is not yet clearly established, but may involve complement activation after binding of the mAb to lipopolysaccharide (LPS), followed by endotoxin clearance involving complement receptors on erythrocytes, and then elimination by Kupfer cells. Phase I clinical trials demonstrated that HA-1A was well tolerated. A fairly large-scale pivotal clinical trial showed that the mAb benefits subpopulations of patients differently, but efficacy (in terms of reduced mortality) seemed evident and this was supported by data derived from a 'compassionate' trial. A large (6000–7000 patients), placebo-controlled trial, which should clarify efficacy issues, is underway in the USA.

S. Ackerman (Xoma, Berkeley, CA, USA) presented data on a well-characterized mouse monoclonal IgM to lipid A (E-5), which appeared to have similar antigen-binding characteristics to HA-1A. Clinical-trial data indicated that some groups of patients responded to therapy, but selection criteria for efficacy were difficult to define. Results showed that treatment with E-5, combined with antibiotic therapy, improved patient survival compared with antibiotic treatment alone. Efficacy was significant in patients with Gram-negative sepsis, but not in shock, whereas those patients who did not have Gram-negative sepsis, or were already in shock, did not appear to respond. Both speakers emphasized the considerable problems in evaluating mAb therapy (and particularly patient responses) in this difficult, but obviously very serious clinical indication.

M. Bodmer (Celltech, Berks., UK) presented pre-clinical data on a tumour necrosis factor α (TNF-α) mAb (CDP571), produced by 'humanizing' a mouse mAb specific for the antigen. Inserting the murine antigen-recognition regions into the human IgG4 structure produced a 'grafted' mAb with binding characteristics similar to those of the original murine mAb. Animal studies suggested that the mAb was able to neutralize TNF-α potency in pyrexic rabbits and could also produce advantageous effects in a primate sepsis model. The immunogenicity of the humanized mAb in primates was markedly reduced compared with the murine parent mAb.

Immunosuppression

The use of mAbs, and potential targets, for inducing immunosuppression to prevent transplant rejection was introduced by R. Thorpe (NIBSC). An in-depth assessment of the clinical use of the well-known mouse CD3 mAb, OKT3, was presented by T. Haverty (R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ, USA). A large body of data derived from several clinical trials is now available, indicating that OKT3 is beneficial to kidney transplant patients, although some adverse reactions have been observed. The mitogenicity of OKT3 and concomitant cytokine release may or may not be related to efficacy, or the adverse effects observed (cytokine-release syndrome). Promising preliminary data using OKT4a (a CD4 mouse mAb) in pre-clinical trials was also presented. The role of cytokine release by lymphocyte-cell-surface antigen-specific mAbs relating to induction of immunosuppression was considered, and data from clinical trials with non-mitogenic CD3 mAbs is eagerly awaited.

D. Tyrell (Cantab Pharmaceuticals, Cambridge, UK) described pre-clinical results obtained using a pair of rat mAbs (YTH 24.5 and 54.12) specific for CD45. Although the individual mAbs fixed complement poorly, the antibodies potently activated complement when mixed; the mAb mixture was shown to be lytic for dendritic cells and other cell types. The results of two clinical-research studies suggested that the cocktail of mAbs showed considerable clinical promise. Initial production was from ascitic fluid, but this had been changed to *in vitro* preparation, largely because of advantages in consistency of production. Clinical trials with these antibodies involving *in vitro* perfusion of donor kidneys is planned, although future *in vivo* use is also a possibility.

P. Amlot (Royal Free Hospital, London, UK) summarized clinical data obtained using two chimaeric mAbs against the CD7 and CD25 antigens that are expressed on T lymphocytes following activation. Anti-CD7 mAbs 'modulate' the CD7 antigen from the cell surface,

*The meeting 'Monoclonal Antibodies: Clinical and Regulatory Issues', organized by IBC Technical Services Ltd, was held in London, UK, 17–19 November 1992.

and may thereby impair T-cell interactions and also kill some T lymphocytes. The anti-CD25 mAbs block binding of IL-2 to its receptor, and thus inhibit proliferative effects due to the cytokine. The chimaeric mAbs were produced by splicing the heavy- and light-chain regions from specific mouse mAbs to the human γ_1 and κ constant regions, respectively. Intravenous administration of either mAb to humans during preclinical studies was well tolerated, and trials have been carried out to assess efficacy in reducing the incidence of renal transplant rejection. Both mAbs showed an extended half-life [two to five days for anti-CD7 (SDZCHH380) and 13 days for anti-CD25 (SDZCH1621)] compared with the murine 'parents'. A very low incidence of immune response to either antibody occurred in recipients, and the trials showed that neither mAb increased the incidence of infections in patients receiving therapy. The efficacy of mAb SDZCHH380 (anti-CD7) seemed promising in the phase I clinical study, but the phase III results appeared to be less pronounced. The phase I trial with SDZCH1621 (anti-CD25) indicated that recipients experienced a reduced incidence of transplant rejection. Phase III placebo-controlled trials with this mAb are about to begin.

Cancer therapy

C. Reynolds [National Cancer Institute (NCI), Bethesda, MD, USA] introduced the use of mAbs for cancer therapy and tumour imaging, and reviewed clinical-trial approaches, goals and data obtained through the mAb programme at NCI. More than 50 mAbs are, at present, under investigation for indications including leukaemia/lymphoma, melanoma and colorectal, breast and lung carcinomas. Selection criteria, specified for clinical development of antibodies, have led to a range of conjugated, fragmented and hybrid antibody molecules being evaluated. Trial results showed that some mAbs were clearly useful for treatment/diagnosis, but a range of side effects and toxicities associated with *in vivo* use could limit the value of these approaches. The potential for using humanized mAbs, combination therapy (mAb/mAb and mAb/cytokine, or chemotherapeutic), bifunctional mAbs and also alternative routes of administration, decrease unwanted side-effects were discussed. NCI is actively pursuing procedures to improve all aspects of the clinical use of mAbs in humans.

R. Begent (Royal Free Hospital) reviewed current work on tumour imaging, and cancer therapy with conjugated mAbs, focusing on the use of radioisotope conjugated mAbs specific for tumour-related antigens [such as carcinoembryonic (CEA) antigen]. Most studies use the β-emitting isotopes ^{131}I and ^{90}Y. However, other long-path-length β-emitters can be used, and at least one trial has been carried out using the α-emitter ^{211}astatine. The results of trials with various antibodies seemed generally encouraging. For therapy, repeated dosing is almost always required, and this can result in an anti-globulin response. Poor tumour penetration could be at least partially resolved by using mAb fragments, and the anti-globulin response minimized by the use of chimaeric mAbs, or combination therapy with immunosuppressives such as cyclosporin A. The tumour : blood ratio is very important and Fabs or single-domain antibodies (dAbs) can perform better than intact mAbs in this respect. For imaging, rapid clearance of the mAb from the circulation is imperative and including an anti-mAb antibody can improve clearance without significantly affecting binding to the tumour target. Refinements such as combination therapy and local administration of mAb can be beneficial.

Bone-marrow transplantation (BMT) is probably the best available treatment for preventing relapses following combination chemotherapy for leukaemia. However, graft versus host disease is a major problem with BMT and immunosuppressives such as cyclosporin A or mAbs must be used to prevent it. Data on the manipulation of bone marrow were presented (M. Hamon; Royal Free Hospital), showing the effects of the *in vitro* use of anti-CD6, CD7, CD8 antibodies with complement; an anti-CD5–ricin A-chain conjugate; an IgM anti-CDW52 mAb (and some other combinations) with donor serum for T-cell depletion of bone marrow. The use of anti-CD19 mAbs in B cell acute lymphoblastic leukaemia also seems promising.

Quality control and regulatory issues

The final session of the conference related topics. J. Purves [Medicines Control Agency (MCA), London, UK] discussed the 'requirements' necessary for licensing mAbs for clinical use and, in particular, obtaining a marketing authorization in the European Community (EC). Official EC policy in this area is covered by a number of legal Directives, and published guidelines outline approaches required at a more advisory level. Guideline documents which may be useful for licensing mAbs are 'Production and quality control of mAbs of murine origin'; 'Production and quality control of mAbs of human origin'; 'Pre-clinical safety testing of medicinal products derived for biotechnology'; and 'Production and quality control of medicinal products derived by recombinant-DNA technology'. Some of these documents were prepared some time ago and are in the process of being updated. It is essential that companies intending to submit documentation to support licensing of mAb products enter into discussions with the licensing authorities at a very early stage and continue the dialogue throughout the regulatory process. Details of the compilation of such documentation (and, in particular, the importance of the 'Expert Report') was outlined.

K. Stein [Center for Biologics Evaluation and Research, Food and Drug Administration (FDA), USA] provided a US orientated version of regulatory issues relating to mAbs The necessity of carefully characterizing the epitope recognized by the mAb was stressed, and the importance of negative controls in such studies was highlighted. Functional studies of mAbs can be crucial, and development of valid potency assays for the product, particularly those which correlate with clinical efficacy, was considered very important. Clinical trials must be designed so that they yield meaningful results that are valid for the intended application. Problems with ill-defined endpoints, identification of target populations, analytical plan and statistical analysis had arisen for some mAbs. The FDA has produced 'Points to Consider' documents specifically relating to mAb licensing applications, which cover all aspects of testing required for licensing – a new document is in preparation.

Using immunochemical procedures such as immunochemistry, immunoprecipitation, and immuno-

showed that cross-reactions with mAbs can occur. In some cases, it was possible to identify the molecular basis of the reaction. Unexpected cross-reactions were found more frequently with IgM mAbs than IgG antibodies, often reacting with intracellular, particularly cytoskeletal, structures such as intermediate filaments. Others showed a more diverse cross reactivity; several reacted strongly with smooth muscle, and at least one (raised against the Rhesus D antigen) seemed to bind to actin or an actin-associated molecule. Such studies can reveal unexpected cross-reactivities that were not an intended property of the mAb. Whether these may cause problems with the *in vivo* use of the antibody in humans is not yet known, but they should at least be borne in mind when pre-clinical and clinical studies are designed and analysed.

J. Ostove (Microbiological Associates Inc., Rockville, MD, USA) provided an extensive review of ten years of testing ~700 cell lines (58% of these were hybridomas) for adventitious agents, and in some cases, other characteristics. Such testing was usually carried out as part of the requirements to meet FDA and/or EC 'Points to Consider' or guideline documents relating to license applications. Schedules were designed on a case-by-case basis and included tests for sterility, mycoplasmas, viruses [including mouse or rat antibody production tests (MAP or RAP testing)] and especially retroviruses, as well as isoenzyme analysis, karyotyping and tumourgenicity testing. The value of the latter two tests was questionable, especially for hybridomas and transformed lymphoblastoid lines. Results showed that <1% of the cell lines studied had viral contaminants detectable by 'classical methods', although a range of viruses including lymphocytic choriomeningitis and Sendai viruses had been found using MAP testing. Bovine diarrhoea virus had been detected in production lines, and ~4% of lines were contaminated with mycoplasmas. Endogenous retroviral contamination is still a contentious issue; most hybridoma lines are affected and some cell culture supernatants contained as many as 10^{11} viral particles per millilitre. Extensive *in vivo* clinical experience with products derived from such lines suggested that they were safe; however, a recent report has shown that similar murine viruses can cause lymphomas in primates either directly, or indirectly as a 'helper' virus.

Overall, the consensus of the meeting was that mAbs are useful therapeutic and *in vivo* diagnostic agents. The next few years should provide conclusive data that, at least for some clinical indications, the 'magic bullet' may at last prove a feasible option.

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Therapeutic antibodies – the coming of age

meeting report

Progress towards the provision of effective monoclonal antibodies (mAbs) for therapy was the subject of two consecutive conferences held recently* in San Diego. The separation of the topic into two conferences, entitled 'Antibody Engineering – New Technology and Application Implications', and 'Commercializing Human Monoclonal Antibodies' – seemed at the outset, even to conference addicts, to be aimed more at increasing fee income, rather than dealing efficiently with this important topic. Indeed, this turned out to be the case, as the gems of the conference became lost in repetition. Therefore, for the purpose of this summary, both conferences have been considered as a single entity.

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Antibodies in the clinic

There is widespread acceptance that there is little future for the use of rodent mAbs for *in vivo* human therapy. This was further emphasized at the conference, by the absence of new clinical data. There are three problems with the use of rodent mAbs: (1) very short half-life; (2) poor recognition of rodent immunoglobulin (Ig) constant (C) regions by human effector functions; and (3) the human immune response against murine proteins (HAMA response). Four approaches to overcoming these deficiencies formed the basis of the conferences.

The genetic engineering approach of converting murine monoclonals into mouse/human chimaerics with murine variable (V) regions and human C-regions – as the earliest of the new technologies – has given rise to several products currently in clinical trial. Data from these trials were summarized by A. LoBuglio (University of Alabama, Birmingham, AL, USA), and J. Woody (Centocor, Malvern, PA, USA), and, in essence, confirmed the conclusions drawn from the more limited data available at the previous year's meeting. There is a wide diversity in results from different studies and with different patient populations, but generally, the half-lifes of the chimaeric antibodies are higher than those of the murine antibodies (chimaeric 100 h; murine 15 h), and the HAMA response is greatly reduced. However, the residual HAMA response to chimaeric antibodies is mainly anti-idiotypic, therefore repeated dosing is ineffective. However, an important conclusion, drawn from all the available data, is that chimaeric antibodies are non-toxic. This gives researchers great confidence that the newer technologies generating more humanized antibodies will lead to the availability of effective therapeutics.

Preliminary clinical results with a humanized antibody (see below)

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against the interleukin 2 (IL-2) receptor (C. Queen, Protein Design Labs Inc., Mountain View, CA, USA) suggested the absence of a HAMA response, but these patients, as graft recipients, are immunosuppressed. By the time of the next meeting (December 1993), the major expectation is that clinical data from the use of at least four humanized mAbs may be available.

Making humanized antibody therapeutics

A major part of both conferences was devoted to the protein engineering of rodent antibodies to provide 'humanized' antibodies; the use of phage immunoglobulin repertoires to identify human antibody fragments directed against target antigens; and improving methodologies to immortalize cell lines producing human mAbs.

Humanized antibodies

The complementary determining region (CDR) grafting technique, initially described by G. Winter and colleagues (reviewed in Ref. 1), is recognized as the major method for converting a rodent mAb into a human therapeutic antibody. It is likely that ~50 mAbs have been 'humanized' by various laboratories around the world, and a number of poster presentations from commercial laboratories stressed this point (including Genentech, Boehringer–Ingelheim, Sandoz, Protein Design Labs Inc., Scotgen Ltd, Bristol–Myers Squibb and Chugai Pharmaceutical Co.). This method involves the transfer of the CDRs from the heavy (H) and light (L) V-chains of the rodent Ig into a human V-domain, followed by the replacement of some human residues in the framework regions with their murine counterparts.

The method of 'homology matching' was described by Queen and is based upon selecting the human H- and L-chain V-genes which resemble most closely the donor rodent antibody in linear amino acid sequence. Eduardo Padlan (NIH, Bethesda, MD, USA) described how his detailed analysis of the structure of different antibodies can be used to identify those amino acid residues in the framework regions which interact with CDRs. These methodologies have been commonly used to date, although W. Harris (Scotgen Ltd, Aberdeen, UK) pointed out that this approach had resulted in the use of a wide variety of human frameworks, and the resulting 'humanized' antibodies had a large variation in the number of murine residues substituted in the framework regions. He described the alternative approach of using a fixed human framework within which the minimal possible number of residues were changed, and presented data which suggested that this approach provides a series of products that is not only more homogenous, but also more 'human'. He also described new attempts to obtain immunologically silent molecules by using human germ-line frameworks to provide humanized 'virgin' antibodies.

Phage repertoires

The preparation of bacteriophage libraries displaying human Ig repertoires is clearly now of general applicability. Again, much of the conference concentrated upon the differences in approach taken by the individual groups. David Chiswell (Cambridge Antibody Technologies, Cambridge, UK) and J. Marks (Medical Research Council, London, UK) described the display of Fab fragments as fusion products of gene III of phage fd, and demonstrated that Fab fragments against a broad range of different antibodies can be isolated from the same human library. On the other hand, R. Schopes (Stratacyte Corp., La Jolla, CA, USA) and S. Zebedee (R. W. Johnston, Pharmaceutical Research Institute, San Diego, CA, USA) described the use of M13 phage to display single-chain Fv fragments to isolate a range of Fv fragments directed against infectious diseases.

Antibody fragments and bifunctional antibodies

A number of presentations emphasized the greater potential therapeutic value of single-chain Fv or Fab fragments to target tumours (S. Kashmiri, National Cancer Institute, Bethesda, MD, USA; A. Epstein, University of Southern California, Los Angeles, CA, USA). Data from *in vitro* studies emphasized the potential of bifunctional antibodies using Fab or ScFv covalently linked to T-cell antigens (P. Carter, Genentech Inc., South San Francisco, CA, USA), toxins (R. Bird, Molecular Oncology Inc., Gaithersburg, MD, USA), vasoactive peptides (A. Epstein, University of Southern California, Los Angeles, CA, USA), or interleukins (P. Savage, Antisoma Ltd, London, UK). The major problem in getting these products to clinical trial, is the inability to synthesize them in large quantities, and the loss of binding that is often associated with these monovalent fragments. To this end, A. Plunkthun (Max Planck Institute, Martinsried, Germany) described his successful work in directly expressing bivalent mini-antibodies (i.e. single-chain Fv) by linking them through amphipathic helices, and M. Whitlow (Enzon Inc., S. Plainfield, NJ, USA) showed that certain single-chain linkers encourage the self-assembly of bivalent molecules in *E. coli*.

Possibly the most important advance presented at this conference, was the demonstration that a single-chain T-cell receptor fragment, comprising only the V_α and V_β chains can be expressed successfully, and that this molecule effectively competes for binding to MHC-presented peptide (D. Segal, National Cancer Institute, Bethesda, MD, USA). Many immunologists still hold the prejudice that T-cell receptor interactions are complex, and therefore beyond molecular analysis and manipulation.

Human monoclonal antibodies

Exciting though the molecular biological developments are, there remains a strong feeling that the most effective and immunologically silent mAbs will be those which have evolved naturally *in vivo*. Hence, there is continuing effort to improve methods for rescuing B cells expressing high-affinity human mAbs. The most significant progress has been in the use of severe combined immunodeficiency (SCID) mice that have been injected with human peripheral blood lymphocytes and then subjected to antigen stimulation. Subsequent rescue of these B cells by Epstein Barr virus (EBV) transformation (C. Borrebaeck, University of Lund, Lund, Sweden), or with phage library repertoires (M. Duchosal, Scripps Research Institute, La Jolla, CA, USA) offers a new methodology for obtaining human mAbs.

Other avenues

The conference also highlighted a number of topics which do not seem to have progressed in the past year. These include immunoadhesins, catalytic antibodies, molecular recognition units (MRUs), small peptides derived from CDRs, long-term maintenance of human lymphocyte

cultures and EBV transformation methodologies.

Separating fact from fiction

This conference is the major annual meeting in the field of therapeutic monoclonals and is a 'must' for researchers in this field. It should now progress past methodological descriptions and the need for every sponsor to be given an opportunity to make a presentation. It was tedious to listen to six descriptions of phage technology, and five descriptions of how to construct a single-chain antibody. However, we look forward to the next conference, to continue the progress in separating fact from fiction and to indicate those technologies and targets which are truly leading to the commercialization of mAbs.

Reference

1 Gussow, D. and Seeman, G. (1991) *Methods Enzymol.* 203, 99–121

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focus

Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics

Jim A. Field, Ed de Jong, Gumersindo Feijoo-Costa and Jan A. M. de Bont

Woody tissues are composed mainly of three biopolymers: cellulose; hemicellulose; and lignin. Lignin, a highly irregular aromatic polymer which serves to provide strength and structure to the tissue, is synthesized in plants by a random peroxidase-catalysed polymerization of substituted *p*-hydroxy-cinnamyl alcohols. Only a few groups of microorganisms are capable of degrading complex lignin polymers, and they are best exemplified by the white-rot fungi, which cause the greatest degree of mineralization. The white-rot fungus *Phanerochaete chrysosporium* has been used extensively as a model organism to study the physiological requirements and enzymes required for lignin biodegradation (ligninolysis). Lignin cannot be degraded as a sole source of carbon and energy, and ligninolysis only occurs when other readily biodegradable substrates are available; *P. chrysosporium* initiates ligninolysis only after primary growth has ceased due to carbon, nitrogen or sulfur limitation[1,2]. The physiological importance of lignin biodegradation is destruction of the lignin matrix so that the microorganisms can gain better access to the real substrates; hemicellulose and cellulose.

The extracellular machinery involved in lignin degradation by *P. chrysosporium* is composed of lignin peroxidases (LiPs) and manganese-dependent peroxidases (MnPs), as well as H_2O_2-producing oxidases (Fig. 1). Lignin peroxidases can abstract one electron from a non-phenolic moiety of the lignin molecule, thus creating a cation radical[4] which in turn initiates a random oxidative chemical reaction that finally results in the oxygenation and depolymerization of lignin. Veratryl alcohol, a metabolite synthesized *de novo*, has an important role in stabilizing LiP against inactivation by H_2O_2 (Ref. 5). Manganese-dependent peroxidases function by oxidizing Mn(II) to Mn(III). Mn(III) behaves as a low-molecular-weight mediator that can diffuse to remote regions of the lignin molecule and initiate the oxidation process[6].

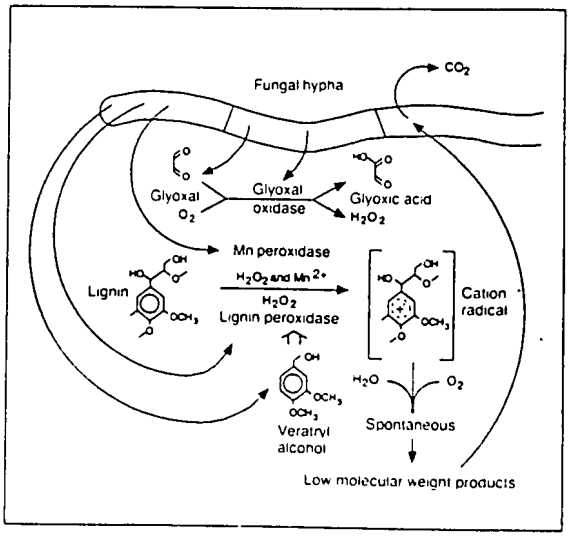

Figure 1

The ligninolytic system of *Phanerochaete chrysosporium*. Redrawn from Ref. 3.

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